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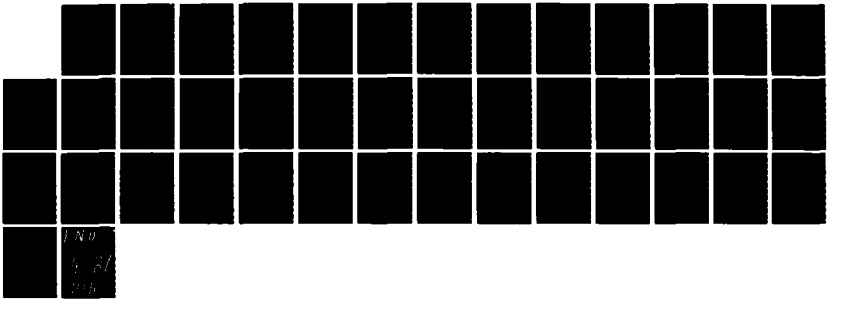

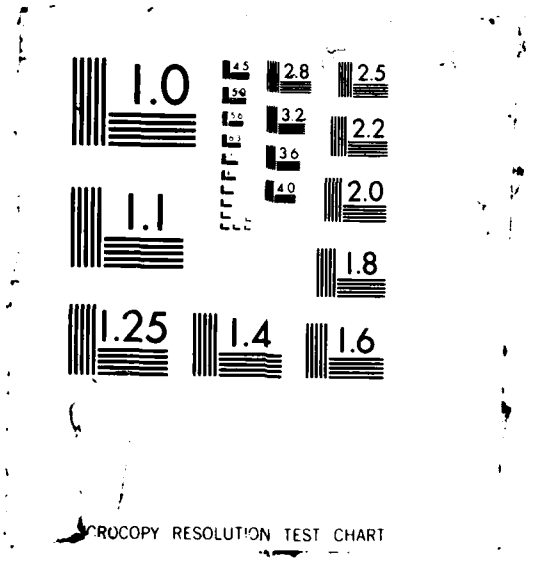
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Two model systems were used to examine the phenomenon of metabolic cooperation between cells. The recovery of ARL-TG^r cells in the presence of hepatocytes and 6-thioguanine was examined. Perfluorodecanoic acid and 2, 2' 4, 4' 5, 5' hexachlorobiphenyl have significant effects on metabolic cooperation between cells; implying that these chemicals interfere with gap junction function and hence interrupt cell-cell communication. These results were confirmed in autoradiographic studies in which the transfer of ^{3}H uridine between donor and recipient cells was examined. If these compounds inhibit cell-cell communication in vivo, then that may be one mechanism by which the toxic effect is exerted.

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"Mechanisms of Cellular Membrane Effects of TCDD, Selected Perfluorinated Acids and Polyhalogenated Aromatic Hydrocarbons"

Submitted To:

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I. INTRODUCTION AND BACKGROUND

Chlorinated dibenzo-p-dioxins are a group of chemical compounds which are among the most toxic and hazardous pollutants in the environment. The most biologically active and most widely studied form is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The major clinical feature of a single lethal dose of TCDD across all animal species is a progressive weight loss and general deterioration of the animal with death occuring in a few weeks or a few months (1). Gross examination and histopathology have not, to date, provided sufficient information to direct biochemical investigations. The major pathologic effect of TCDD in mice and rats is degeneration of the liver. However, the degree of injury to the liver does not appear to be sufficient to be the cause of death.

Recent evidence suggests that perfluorinated carboxylic acids of chain length 10 or greater have toxic effects in vivo strikingly similar to those caused by TCDD (2). It has also been proposed that polyhalogenated aromatic hydrocarbons (PHAH) lacking halogen at the bridgehead position may also have toxic effects similar to those of TCDD ((3) and M.E. Andersen, personal communication).

The extreme lethality of TCDD suggests that it is probably affecting some fundamental process in animal cells leading to a general dysfunction of these cells. A number of biochemical endpoints have been examined to determine the mechanism of TCDD toxicity (for review see Neal, et. al., 1979 (1)). To date, none of these avenues of approach have yielded definitive answers.

One proposed mechanism of TCDD toxicity is that TCDD binds to a receptor in the cytosol fraction of mammalian cells and is transferred to the nucleus where it increases or decreases the synthesis of a critical protein(s) or enzyme(s) (4). This increase or decrease in critical protein or enzyme levels leads to a fundamental change in cellular metabolism leading eventually to cell death.

An alternate mechanism for TCDD-(and similar acting compounds) induced toxicity is the concept that altered membrane function is the underlying biochemical mechanism responsible for toxicity (3). Fatty acid analysis of livers from rats treated with perfluoro-n-decanoic acid (PFDA) showed a decrease in stearic acid and an increase in 18 carbon unsaturates relative to controls (3). Andersen et. al., (3) proposed that PFDA and TCDD interfere with fatty acid metabolism leading to an increase in unsaturation. The increased unsaturation leads to excessive membrane fluidity and impaired membrane function. Therefore, MBA proposes herein to investigate the effects of TCDD, PHAH and perfluorinated acids (PFA) on mammalian cell membrane function by examing the phenomenon of metabolic cooperation.

Metabolic cooperation, the phenomenon of metabolite exchange across intercellular contacts, was first detected between cultured mammalian cell by Subak-Sharpe et. al., (5). It is commonly believed that metabolic cooperation is mediated by permeable, intercellular gap junctions (6). Examination of a variety of cell types has shown that the ability to engage in metabolic cooperation correlates directly with the capacity for

intercellular exchange of ions and fluorescent tracer molecules and with the ability to form gap junctions (7,8).

Metabolic cooperation has usually been detected as the phenotypic modification of mutant cells when in contact with wild-type cells (5). The mutant cells lack activity in certain enzymes of the purine or pyrimidine salvage pathways. For example, cells lacking hypoxanthine-guanine phosphoribosyl transferase (HGPRT) activity fail to incorporate 3H-hypoxanthine into nucleic acid when growing in isolation, but they may do so when grown in contact with wild-type cells (5). This observation has been explained most readily as an intercellular passage, through gap junctions, of 3H-labelled nucleotides.

It may be that the process of cell growth and metabolic regulation occur through cell-to-cell transfer of regulatory chemicals or electrical signals. Thus, the process of metabolic cooperation in cell cultures may simulate the intercellular communication which takes place in vivo.

Two approaches have been developed to determine the effects of compounds on metabolic cooperation. Trosko and co-workers (9,10) have developed a system using the recovery of mutant cells as a marker for metabolic cooperation. In brief, when an HGPRT-cell is induced in a population of wild-type $HGPRT^+$ cells, the wild-type cells can transport the toxic metabolic (phosphorylated 6-thioguanine) formed in the presence of the $HGPRT^+$ enzyme and 6-thioguanine (TG) to the $HGPRT^-$ cells, thus killing mutant cells. Trosko and co-workers have used an $HGPRT^-$ and $HGPRT^+$ V79 Chinese Hamster cell line. A mixed population of

$HGPRT^+$ and $HGPRT^-$ cells is seeded in a culture dish and allowed to attach. TG and the test chemical are then added to the dishes. The cells are grown for three to four days and the medium is changed. The test chemical is removed and growth is continued in the selective medium (i.e., in the presence of TG). After four days, the medium is decanted, the dish rinsed with saline, air dried, fixed with ethanol and stained with Giemsa. The resulting colonies are scored visually. To date, Trosko and co-workers have observed an inhibition of metabolic cooperation after cells have been treated with a number of agents e.g. DDT, a polybrominated biphenyl (Firemaster BP-6) and phorbol myristate acetate (9). The effect of PHAH on metabolic cooperation has been studied by Tsushimoto et al. (11). The authors found that the degree of cytotoxicity of polybrominated biphenyls (PBB) is inversely related to the degree of bromination in the ortho position of the phenyl rings. Thus the 2,2',4,4'5,5'-hexabromobiphenyl (HBB) is less cytotoxic than the 3,3'4,4'5,5'-HBB. This finding is in agreement with in vivo studies (12). However, the study showed that only the less cytotoxic congeners inhibited metabolic cooperation. The authors suggested that the PBBs, as a family, act as tumor promoters and that tumor promoters induce their cellular responses by their membrane-perturbing properties.

An alternate protocol has been developed by Williams and co-workers (13). In this protocol, a mixed population of HGPRT- adult rat liver epithelial cell (ARL) line No. 14 and freshly isolated rat hepatocytes is seeded and allowed to attach. The

procedure is then similar to that of Trokso et al, outlined above. A number of chemicals e.g. DDT have shown dose-dependent inhibition of intercellular communication in this assay system (11). We have used the sytem developed by Williams as a model system for the research reported herein.

An alternate approach to examining the effect of TCDD and PFA on cell membrane has been used by Andersen et al. (14). They used an L5178Y mouse lymphoma cell line and measured growth in suspension and growth in soft agar after treatment with the compounds. Growth in suspension was not markedly affected by any of the compounds. However, the perfluorinated acids with chain length greater than 8, induced a loss of clone-forming ability. TCDD altered clone morphology but did not induce a loss of clone-forming ability. The authors proposed that subtle changes may have occured in cell membranes to inhibit growth of cells when maintained in close contact. However, further investigation of this phenomenon could not be attempted as the cell line was lost. We have repeated the studies described above with the same cell line.

Objectives

The objective of the first year of the contract was to examine the mechanism of cellular membrane effects of six compounds. The proposed compounds were 2,3,7,8 tetrachloro dibenzo-p-dioxin (TCDD); dioxin; perfluoro-n-decanoic acid (PFDA); perfluoro-n-octanoic acid (PFOA); $3,3^1,4,4^1,5,5^1$ hexachlorobiphenyl (345PCB) and $2,2^1,4,4^1,5,5^1$ hexachlorobiphenyl (245 PCB). Two experimental approaches were adopted. In the

first approach, the effect of these compounds on the inhibition of metabolic cooperation between cells was examined. The principal underlying this approach is that the in vitro phenomenon of metabolic cooperation may simulate intercellular communication in vivo. In the second approach, the effect of the compounds on the colony forming ability of L5178Y mouse lymphoma cells was examined. In the second year of the contract, the effect of the compounds on metabolic cooperation was further investigated. In addition, the effect of the compounds on cell membrane function was examined by autoradiography.

Materials and Methods

The experimental protocol for assaying inhibition of metabolic cooperation between cells was essentially the same as that published by Williams, et al, (11) with the modifications discussed in the results section. The experimental protocol for determining the effect on colony-forming ability was essentially the same as that described by Andersen et al, (14).

a) Experimental Protocol for Metabolic Cooperation

(1) Cell Cultures

The 6-thioguanine resistant (TF^r) strain of adult rat liver epithelial cells designated ARL-14 (obtained originally from Dr. G. Williams) was derived by selecting HGPRT deficient mutants after exposure to TG. The TG^r cell line is deficient in HGPRT activity.

Primary rat hepatocytes were obtained using the method of Williams, et al, (15). Briefly, the method involves

perfusing a rat liver with an EGTA (Ethyleneglycol-bis-[2-eminoethyl ether]-tetracetic acid) solution followed by a collagenase solution. The temperature and pH of the perfusates are strictly controlled. The liver is excised from the animal, placed in a 100 mm dish and the capsule opened at various points. Cells are shaken loose and the liver is then combed to remove the remaining cells. The cells are centrifuged, resuspended in Williams Medium E (WME) and a viable count using trypan blue is made.

(2) Metabolic Cooperation Studies

Initial range-finding experiments were performed using ARL-14-TG^r cells to determine non-toxic doses of the compounds to be tested. Valid results in the metabolic cooperation studies are obtained only when there is no appreciable cytotoxicity.

ARL-14-TG^r cells were plated at a density of 20 cells/cm^2 in WME buffered with 10mM HEPES, supplemented with 10mU insulin/ml, 2mM L-glutamine, 50 ug/ml gentamycin and 10 percent calf serum (WMES). Twenty-four hours later, freshly isolated hepatocytes were plated onto the same flasks and allowed to attach for two hours. Figure 1 presents a flow chart for the experiments.

For each chemical concentration tested, six control flasks were used to determine the colony-forming ability of ARL-14-TG^r cells grown in the presence of the test chemical of TG. The control flasks were handled identically to the treatment flasks with the exclusion of the hepatocytes. A positive control

chemical, DDT and a negative solvent control were included as internal checks of the experiment.

b) Experimental Protocol for Measuring the Effect of PFA, and PHAH on Colony-Forming Ability in L5178Y Cells

(1) Cell Line

L5178Y cells were obtained from Dr. C.F. Arlett, MRC Cell Mutation Unit, Brighton, England. The routine methods for maintenance of L5178Y cells and the soft agar cloning technique were as described elsewhere (16).

(2) Determination of Colony-Forming Ability After Treatment

L5178Y cells were tested in suspension for 24 hours with doses of perfluorinated acids ranging from 0.01 ug/ml to 1 mg/ml. For PHAH, a preliminary toxicity test was conducted to determine the optimal time of exposure and concentration of PHAH to use in the studies.

At the end of the treatment period, cells were centrifuged, washed twice in Fischer's medium containing 10 percent horse serum ($F_{10}P$) and resuspended in $F_{10}P$. The cells were counted, readjusted to a cell density of 0.3×10^6 cells/ml in 10 ml, diluted to give a final density of 200 cells/15 cm plate and plated in Fischer's medium containing 20 percent horse serum and 0.34 percent purified agar. The plates were incubated for 10 days in a humidified CO_2 incubator and colony growth was then determined.

Determination of Gap Junction Formation

a) Uptake of 3H-uridine

The basis for these experiments lies in the observation that TG^r cells fail to incorporate 3H-hypoxanthine. However, in the presence of wild-type cells, the mutant cells are able to incorporate 3H-hypoxanthine. It has been shown that a nucleotide or a nucleotide derivative is transported from the wild-type cell to the mutant, thus bypassing the enzyme block in the latter (7).

ARL-14-TG^r cells and freshly isolated hepatocytes were grown as outlined above, except that the medium contains 3H-uridine and the cells were grown on plates. The test chemical was added to the plates and incubated for 24 hours. The medium was removed, the cells washed twice in medium containing 10^{-4} cold uridine, fixed with 2 percent glutaraldehyde, rinsed in 10 percent trichloracetic acid, and covered with photographic emulsion. Autoradiographic exposures were for five days. Scoring was performed using an automated colony counter with a microscope attachment. Two controls were incorporated in each experiment. One control consisted of untreated hepatocytes and ARL-14-TG^r cells, and the second control comprised hepatocytes and ARL-14-TG^r cells with 1×10^{-5}M DDT.

Results

L5178Y Mouse Lymphoma Cells

The first six months of the contract were devoted to examining the effects of perfluorinated acids and polyhalogenated

aromatic hydrocarbons on the colony-forming ability of L5178Y cells. Two sub clones of this cell line were utilized. One sub clone is designated L5178Y TK+/- and this line was obtained from Dr. D. Clive, Burroughs Wellcome, North Carolina. The second sub clone is designated L5178Y TK+/+ and was obtained from Dr. C. Arlett, MRC Cell Mutation Unit, England. The two cells lines differ markedly in their growth characteristics. L5178Y TK+/- cells tends to associate in clumps of cells and are grown in a shaker incubator to produce better cell suspensions. L5178Y TK+/+ cells grown as a single cell suspension without agitation.

The toxic response of both cell lines after treatment with the perfluorinated acids (perfluoro-n-octanoic acid, 9-H hexadecafluoro-n-nonanoic acid, and perfluoro-n-decanoic acid) was measured. Two different cell media were used in the experiments to determine if the growth milieu of the cells had an effect on clonal or suspension toxicity. Experiments were conducted in Fischer's medium and in McCoy's 5A medium. The same lot of horse serum was used throughout the experiments to eliminate the effect of serum on toxicity.

The results for perfluoro-n-octanoic acid (PFOA) in both cell lines are presented in Table 1. For both cells lines and in both types of media, there was no apparent dissociation of suspension growth from clonal growth. These results are in agreement with results obtained by Andersen, et al (1983). The results for 9-H hexadecafluoro-n-nonanoic acid (9-HFNA) (Table 2) indicate that, for the TK+/- cells there is a reproducible dissociation of suspension growth from colony growth at 100

ug/ml. The effect is not observed at concentrations of 50 ug/ml or less. The effect is also seen at 100 ug/ml in the TK+/+ cells. However, this concentration produces more toxicity in suspension in TK+/+ cells when the responses of the TK+/+ and TK+/- cells are compared. There is some dissociation of colony growth from suspension growth at 50 ug/ml in the TK+/+ cells but not in the TK+/- cells.

The results for the perfluoro-n-decanoic acid (PFDA) are presented in Table 3. A dose-response relationship for toxicity and the ability to form colonies in soft agar was noted in both cell lines. This effect was apparent at 500, 400, 300, 200 and 100 ug/ml. At concentrations of 50 ug/ml or less, there was no difference between suspension growth and colony growth. These results are different from those obtained by Andersen and co-workers. In that study, complete dissociation was observed at 10 ug/ml. We have observed complete dissociation only at concentrations of 200 ug/ml and greater in TK+/+ and at concentrations of 400 ug/ml and greater TK+/- cells.

We have also examined the effect of decanoic acid on TK+/+ cells using McCoys 5A medium in an attempt to reproduce some studies conducted by Andersen, et al. The results are presented in Table 4. There was no apparent dissociation of suspension growth from colony growth. This result is in agreement with results obtained by Andersen and co-workers.

In summary, the results for the perfluorinated acids with chain length of nine or ten indicate that there is some dissociation of colony growth from suspension growth. Medium

type does not affect the toxicity. This would imply that these perfluorinated acids are producing toxicity through a membrane interaction. However, these results are not clear cut as those previously obtained by Andersen, et al. The dissociation appears to occur in the TK+/+ cells at concentrations approximately 20 fold higher than those previously reported.

The optimum exposure time for two polyhalogenated aromatic hydrocarbons (PHAH) was determined. In the presence of an Aroclor induced rat liver S-9, the optimal exposure time is four hours. Longer exposure times were attempted; however, the S-9 mix appears to be toxic to L5178Y cells if the cells are exposed for periods over four hours. In the absence of S-9, the optimum exposure time was determined to be 24 hours. Two PHAH were tested, 2,2',4,4',5,5' hexachlorobiphenyl and 3,3',4,4',5,5' hexachlorobiphenyl. These results indicated that at the maximum soluble concentration of both PHAH (50 ug/ml) there was no significant toxicity in suspension in either TK+/+ or TK+/- cells. The results of the cloning experiments are presented in Tables 5 and 6. Tables 5 and 6 present results of treatment in the presence of induced rat liver S-9. For clarity, the results without activation have not been presented. However, there was no significant difference in toxicity when the induced rat liver S-9 results were compared with non-activated treatments. The results indicate that neither of the PCB congeners produces significant toxicity in L5178Y cells. The two L5178Y cell lines are not different in their response to the congeners and culture conditions (different medium type) have no effect on toxicity.

There is no dissociation of suspension growth from clonal growth by either 345 PCB or 245 PCB in the presence or absence of exogenous metabolic activation.

Metabolic Cooperation Studies

A series of experiments was conducted at the beginning of the study to determine the most suitable concentration of DDT (the positive control compound) to use in subsequent assays. In conjunction with these experiments, we attempted to vary the experimental conditions to improve upon the protocol suggested by Williams, et al (13). Various parameters were altered in the assay, including ARL-TG^r cell density, hepatocyte density, attachment times for freshly isolated hepatocytes, frequency of medium changes during the expression period and length of the expression period. The final protocol is presented in Figure 1. This modified protocol has resulted in significantly enhanced cloning efficiencies in the ARL-TG^r cells. We routinely obtain cloning efficiencies of 45 percent and 49 percent compared with cloning efficiencies of approximately 23 percent reported by Williams and co-workers.

A summary of the results for DDT is presented in Table 7. The results represent approximately ten assays. It is apparent that the survival of ARL-TG^R cells increases with increasing concentrations of DDT until cytotoxic concentrations are reached ($3x10^{-5}$M and $2x10^{-5}$M). The increase in survival of the ARL-TG^R cells is most likely due to the inhibition of metabolic cooperation between the ARL-TG^r cells and the hepatocytes. Thus the toxic derivative of TG is not transferred from the wild-type

cells (hepatocytes) to the resistant cells. We have selected $1x10^{-5}M$ DDT as the optimum concentration for the positive control.

Perfluorinated Acids

The results of the first series of experiments on PFDA are presented in Table 8. The results represent two assays. Table 9 presents the results for a further six assays on PFDA to examine a narrower dose range. Initial experiments with PFDA (Table 8) indicated that the survival of ARL-TG^r cells increased at concentrations of 10 ug/ml. However, at 50 ug/ml PFDA there was a marked cytotoxic effect as evidenced by reduced colony growth in the "no-hepatocyte" flasks. A series of experiments was then initiated to examine concentrations of PFDA in the range of 10 ug/ml to 50 ug/ml. These results are presented in Table 9. There is a clear dose-dependent increase in ARL-TG^r cell recovery at increasing concentrations of PFDA up to 50 ug/ml. 50 ug/ml is cytotoxic. The results indicate the PFDA inhibits metabolic cooperation between cells. This would suggest that at least one component of the in vitro toxicity of PFDA is the ability of PFDA to disrupt intercellular communication.

The first series of experiments on PFOA suggested that this compound has no effect on metabolic cooperation (Table 10). Survival of ARL-TG^R cells in the presence of hepatocytes plus varying concentrations of PFOA was measurably enhanced. There was a slight toxicity noted at 50 ug/ml and experiments were initiated to examine the dose range of 10 ug/ml to 50 ug/ml (Table 11).

The results indicated no significant effect of PFOA on ARL-TG^r cell recovery at any of the doses examined. Hence, PFDA does not inhibit intercellular communication.

We have also examined the effect of decanoic acid on metabolic cooperation. Decanoic acid was selected as a control for the PFDA results. We wanted to determine whether the positive effect noted for PFDA was a function of the fluorine substitution and/or a function of chain length. Comparison of the PFDA results with the results for PFOA would suggest that the fatty acid chain length is important. The results for decanoic acid (Table 12) indicated that there was no significant effect of that compound on metabolic cooperation. The results presented are the mean of two experiments. Decanoic acid was toxic to the cells at a concentration of 100 ug/ml. Thus it appears that the fluorination of the fatty acid chain is an important factor in determining the potential of a chemical to interfere with metabolic cooperation.

Polychlorinated Biphenyls

The results of the experiments with 2,4,5 PCB are presented in Table 13. (Results are the mean of three experiments.) There is a clear positive response at concentrations of 1.0 ug/ml and 2.5 ug/ml. Survival of ARL-TG^r cells in the presence of hepatocytes at these concentrations was significantly higher than that of the untreated control cells. The results indicate that 2,4,5 PCB inhibits metabolic activation between cells. This result is in agreement with that obtained for 2,2',4,4'5,5' hexabromobiphenyl by Tsushimoto et al (11).

The results for 3,3',4,4',5,5' PCB are presented in Table 14 (mean of three experiments). It is clear that 3,4,5 PCB is more toxic to ARL-TG^r cells than 2,4,5 PCB. The dose response curve for toxicity is particularly steep for the 3,4,5 PCB congener. 1.0 ug/ml 3,4,5 PCB produces approximately 40 percent cell survival, while a concentration of 0.5 ug/ml produces 76 percent cell survival when compared to the solvent controls. Concentrations lower than 0.5 ug/ml had no appreciable effect on survival or metabolic cooperation. We attempted to examine oncentrations in the range of 1.0 ug/ml to 0.5 ug/ml. However, we were unable to select a dose that produced little or no toxicity (data not shown). The results obtained in these studies indicate that 3,4,5 PCB does not inhibit intercellular communication. It should be noted, however, that the steep dose-response curve for toxicity may preclude the selection of appropriate doses for testing.

Determination of Gap Junction Formation

The principle behind the metabolic cooperation assay is that when a TG-sensitive cell (in this case, hepatocytes) is coupled to a TG^r cell, in the presence of G-thioguanine, the TG-sensitive cell will metabolize the drug to a lethal phorphorylated substrate which kills both the sensitive and resistant cell. If chemicals inhibit gap junction function, the lethal metabolite is not transferred to the TG^r cell from the TG^s cell, allowing the TG cell to survive.

We have examined the phenomenon of metabolic cooperation by two methods, recovery of TG^r cells and also by investigating

the transfer of ^{3}H-uridine from doner to recipient cells by autoradiography. For the autoradiographic studies, ARL-TG^{r} cells were labelled for 18 hours with ^{3}H-uridine (10uCi). The recipient cells (hepatocytes) were added, co-cultivated, as described in the Materials and Methods section, fixed and stained. Four compounds were examined using the autoradiographic technique. PFDA and PFOA were examined in addition to 2,4,5 PCB and 3,4,5 PCB. The results for PFDA are presented in Table 15 (mean of three experiments). The results confirm those reported above for cell recovery in the presence of 6-thioguanine. In the solvent control slides, over 80 percent, on average, of the recipient cells, were labelled. In the presence of PFDA, the percentage of labelled recipient cells fell to 10 percent for the highest concentration of PFDA. The response was dose-dependent. In contrast, the results for PFOA treatment of cells (Table 16) did not indicate that PFOA inhibited intercellular communication. All recipient cells were labelled.

For 2,4,5' PCB (Table 17) there was a clear dose-dependent reduction in the percentage of labelled recipient cells. This result would indicate that 2,4,5 PCB effectively inhibits intercellular communication by interfering with gap junction function. These results are in agreement with those obtained in the TG^{r} cell recovery experiments discussed above. The results for 3,4,5 PCB (Table 18) are not as clear cut. There may be some reduction in the percentage of labelled recipient cells at a concentration of 0.5 ug/ml, but the response is not dose dependent. Higher concentrations (1.0 ug/ul and above) were

toxic in the cell recovery experiments and were not attempted in the autoradiographic studies. However, concentrations did not have a significant effect on the percentage of labelled recipient cells.

Conclusion

We have used two model systems to examine the phenomenon of metabolic cooperation between cells. By examining the recovery of ARL-TG^r cells in the presence of hepatocytes and G-thioguanine, we have demonstrated that perfluorodecanoic acid and 2,2'4,4'5,5' hexachlorobiphenyl have significant effects on metabolic cooperation between cells. The implication is that these chemicals interfere with gap junction function and hence interrupt cell-cell communication. These results were confirmed in autoradiographic studies in which the transfer of ^{3}H uridine between donor and recipient cells was examined. If these compounds inhibit cell-cell communication in vivo, then that may be one mechanism by which the toxic effect is exerted.

In contrast, perfluoroctanoic acid and decanoic acid had no effect on metabolic cooperation or the transfer of ^{3}H uridine between cells. It would appear that these compounds do not affect cell-cell communication via gap junctions. The results for 3,3'4,4',5,5' hexachlorobiphenyl are not so clear cut. The compound produces a very steep dose-response curve for toxicity, and it was not possible to select a concentration which was not either completely toxic or completely non-toxic. We did not obtain any significant effect on the recovery of ARL-TG^r cells in

the presence of 3,4,5 PCB. However, we may not have examined the appropriate concentrations. Experiments using the transfer of ^{3}H uridine between cells did indicate some effect on gap-junction function at one dose. The effect was reproducible between experiments but did not appear to be dose-dependent.

In the experiments on colony-forming ability in L5178Y cells, we obtained dissociation of suspension growth and colony growth at concentrations of PFDA of 200 ug/ml and greater in TK+/+ cells and at concentrations of 400 ug/ml and greater in TK+/- cells. None of the other compounds examined produced dissociation of suspension growth and colony growth. The results imply that PFDA has an effect on L5178Y cell membrane.

In conclusion, we have demonstrated that PFDA and 2,4,5 PCB have significant effects on cell membranes and that these compounds disrupt gap-junction function. These results have implications for the mechanism of toxicity of these compounds in vivo.

Abstract and Planned Publications

An abstract was presented on the metabolic cooperation studies at the 25th Annual Meeting of the Society of Toxicology in New Orleans, March 1986. In addition, a manuscript is being prepared on the results of the research for submission to Toxicology and Applied Pharmacology.

Rogers-Back, A.M. and Clarke, J.J. Effect of perfluorinated carboxylic acids and polychlorinated biphenyls on intercellular communication. 25th Annual Meeting of the Society of Toxicology. New Orleans, LA. The Toxicologist 6:39 (1986).

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Figure 1

Protocol for Inhibition of Metabolic Cooperation Between Cells

Day 0: Inoculate T-25 tissue culture flasks with 500 ARL-14-TG^R cells. (6 flasks per treatment level, six negative control flasks, six solvent control flasks and six positive control flasks.

Day 1: Add hepatocytes at a concentration of 1.25×10^6 cells per flask to half the flasks containing ARL cells. Allow 2 hours for hepatocyte attachment. Add test article, positive control and solvent control to appropriate flasks. Incubate for 2 hours, add 6-thioguinine (TG) (final concentration 10 ug/ml) to all flasks.

Day 2: Replace media in all flasks with fresh medium containing TG plus test article (where appropriate).

Day 3: As Day 2

Day 4: Replace media in all flasks with fresh medium containing TG.

Day 10: For flasks containing hepatocytes, pour off medium, rinse, stain and count colonies.

Day 12: For all remaining flasks, pour off medium, rinse stain and count colonies.

Table 3. Toxicity of Perfluoro-n-decanoic acid in L5178Y Mouse Lymphoma Cells

ncentration (ug/ml)	L5178Y TK+/+				L5178Y TK+/-			
	Fischer's		McCoys 5A		Fischer's		McCoys 5A	
	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal
500	cell	lysis	cell	lysis	cell	lysis	cell	lysis
400	17%	0	21%	0	13%	0	15%	0
300	13%	0	23%	0	19%	0	18%	0
200	19%	0	21%	0	15%	0	18%	0
100	45%	38	39%	29%	27%	2%	28%	12%
50	89%	111%	78%	76%	82%	78%	91%	89%
10	88%	113%	89%	95%	102%	120%	94%	98%
5.0	99%	115%	98%	101%	101%	108%	110%	95%
1.0	90%	110%	99%	103%	107%	119%	101%	93%
0.5	98%	108%	101%	110%	105%	107%	102%	97%

Mean of 3 experiments for each data point.

Table 4. Toxicity of Decanoic Acid in L5178Y TK+/+ Cells Grown in McCoys 5A Medium

Concentration (ug/ml)	Suspension	Clonal
1000	cell lysis	
500	no growth	
400	8%	10%
300	13%	12%
200	16%	15%
100	27%	25%
50	66%	46%

Table 5. Toxicity of 2,2',4,4',5,5' hexachlorobiphenyl in L5178Y Mouse Lymphoma Cells

Concentration (ug/ml)	L5178Y TK+/+				L5178Y TK+/-			
	Fischer's		McCoys 5A		Fischer's		McCoys 5A	
	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal
50	87%	86%	91%	92%	84%	86%	91%	90%
25	93%	92%	95%	90%	89%	91%	94%	94%
10	101%	99%	103%	101%	92%	94%	89%	97%
5.0	98%	98%	103%	99%	97%	95%	93%	101%
1.0	102%	101%	105%	99%	97%	98%	97%	98%

Mean of 3 experiments for each data point.

Table 6. Toxicity of 3,3¹,4,4¹,5,5¹ hexachlorobiphenyl in L5178Y Mouse Lymphoma Cells

	L5178Y TK+/+				L5178Y TK+/-			
	Fischer's		McCoys 5A		Fischer's		McCoys 5A	
ncentration (ug/ml)	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal
50	84%	85%	81%	84%	87%	91%	92%	90%
25	93%	91%	89%	93%	95%	97%	91%	92%
10	91%	90%	92%	97%	97%	97%	95%	95%
5.0	92%	93%	95%	99%	101%	99%	94%	97%
1.0	97%	98%	99%	99%	103%	98%	97%	98%

Mean of 3 experiments for each data point.

Table 7. Effect of DDT on Metabolic Cooperation Between ARL-14-TG^R Cells and Freshly Isolated Hepatocytes

Exposure Condition in TG	No. of TG^R Colonies per Flask[1] + 1.25×10^6 HPC[2]	No. HPC[2]
3×10^{-5}M	110	87
2×10^{-5}M	154	91
1×10^{-5}M	378	187
1×10^{-6}M	259	156
1×10^{-7}M	270	188
Solvent Control (DMSO)	225	195
Negative Control	253	193

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 8. Effect of Perfluoro-n-decanoic Acid on Metabolic Cooperation

Exposure Conditions in TG	No. of TG^R Colonies per Flask[1] + 1.25×10^9 HPC[2]	No. HPC[2]
50 ug/ml	139	86
10 ug/ml	256	186
5.0 ug/ml	214	157
2.5 ug/ml	175	161
Solvent Control (DMSO)	207	155
Negative Control	209	132
1×10^{-5}M DDT	349	170

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 9. Effect of Perfluoro-n-decanoic Acid on Metabolic Cooperation

Exposure Condition in TG	No. of TG^R Colonies per Flask[1]	
	+ 1.25×10^6 HPC[2]	No. HPC[2]
50 ug/ml	146	92
40 ug/ml	302	121
30 ug/ml	289	143
20 ug/ml	281	176
10 ug/ul	262	191
Solvent Control (DMSO)	193	156
Negative Control	201	144
1×10^{-5}M DDT	356	172

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 10. Effect of Perfluoro-n-octanoic Acid on Metabolic Cooperation

Exposure Condition In TG	No. of TG^R Colonies per Flask[1] + 1.25×10^6 HPC[2]	No. HPC[2]
50 ug/ml	219	121
10 ug/ml	227	142
5.0 ug/ml	234	149
2.5 ug/ml	243	130
Solvent Control	209	177
Negative control	239	195
1×10^{-5}M DDT	356	197

1= average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 11. Effect of Perfluoro-n-octanoic Acid on Metabolic Cooperation

Exposure Condition in TG	No. of TG^R Colonies per Flask[1] + 1.25×10^6 HPC[2]	No. HPC[2]
50 ug/ml	214	118
40 ug/ml	219	123
30 ug/ml	236	137
20 ug/ml	227	142
10 ug/ml	231	159
Solvent Control (DMSO)	214	182
Negative Control	223	189
1×10^{-5}M DDT	364	201

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 12. Effect of Decanoic Acid on Metabolic Cooperation

Exposure Condition in TG	No. of TG^R Colonies per Flask[1] + 1.25×10^6 HPC^2	No. HPC^2
100 ug/ml	125	76
75 ug/ml	178	122
50 ug/ml	193	158
25 ug/ml	201	169
10 ug/ml	189	171
Solvent Control (DMSO)	197	168
Negative Control	211	159
1×10^{-5}M DDT	347	169

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 13. Effect of 2,2,4,4,5,5 Hexachlorobiphenyl on Metabolic Cooperation

Exposure Condition in TG	No. of TG^R Colonies per Flask[1]	
	+ 1.25×10^0 HPC[2]	No. HPC[2]
5.0 ug/ml	213	139
2.5 ug/ml	381	153
1.0 ug/ml	345	140
0.5 ug/ml	220	144
Solvent Control (DMSO)	246	163
Negative Control	249	157
1×10^{-5}M DDT	375	137

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 14. Effect of 3,3',4,4',5,5' Hexachlorobiphenyl on Metabolic Cooperation

Exposure Condition in TG	No. of TG^R Colonies per Flask[1] + 1.25×10^0 HPC[2]	No. HPC[2]
5.0 ug/ml	63	40
2.5 ug/ml	79	53
1.0 ug/ml	101	63
0.5 ug/ml	191	120
0.25 ug/ml	189	138
0.1 ug/ml	201	152
Solvent Control (DMSO)	210	157
Negative Control	215	178
1×10^{-5}M DDT	306	149

1 = average of 3 flasks per treatment group
2 = HPC = hepatocytes

Table 15. Effect of PFDA on the Transfer of ^{3}H Uridine Between ARL-TG^{r} Cells and Hepatocytes

Concentration (ug/ml)	Number of Cells Scored	Percent Recipients		
		Heavily Labelled	Lightly Labelled	Unlabelled
50	100	12	19	69
40	100	18	21	61
30	100	21	30	49
20	100	21	31	48
10	100	25	35	30
Solvent Control (DMSO)	100	78	15	7
$1x10^{-5}$M DDT	98	7	14	79

Table 16 Effect of PFOA on the Transfer of ^{3}H Uridine Between ARL-TG^{r} Cells and Hepatocytes

Concentration (ug/ml)	Number of Cells Scored	Percent Recipients		
		Heavily Labelled	Lightly Labelled	Unlabelled
50	100	61	15	24
10	100	71	17	12
5.0	100	65	18	17
2.5	100	69	20	11
Solvent Control (DMSO)	100	75	18	7
$1x10^{-5}$M DDT	100	10	17	73

Table 17. Effect of 2,4,5 PCB on the Transfer of ^{3}H Uridine Between ARL-TG^{r} Cells and Hepatocytes

Concentration (uq/ml)	Number of Cells Scored	Percent Recipients		
		Heavily Labelled	Lightly Labelled	Unlabelled
5.0	100	20	18	62
2.5	100	25	21	54
1.0	100	31	23	46
0.5	100	65	20	15
Solvent Control (DMSO)	100	73	20	7
$1x10^{-5}$M DDT	100	12	19	69

Table 18. Effect of 345 PCB on the Transfer of ^{3}H Uridine Between ARL-TG^{r} Cells and Hepatocytes

Concentration (ug/ml)	Number of Cells Scored	Percent Recipients		
		Heavily Labelled	Lightly Labelled	Unlabelled
0.5	100	42	18	40
0.25	100	63	19	18
0.1	100	74	19	7
0.05	100	77	12	11
Solvent Control (DMSO)	100	81	12	7
$1x10^{-5}M$ DDT	100	12	19	69

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